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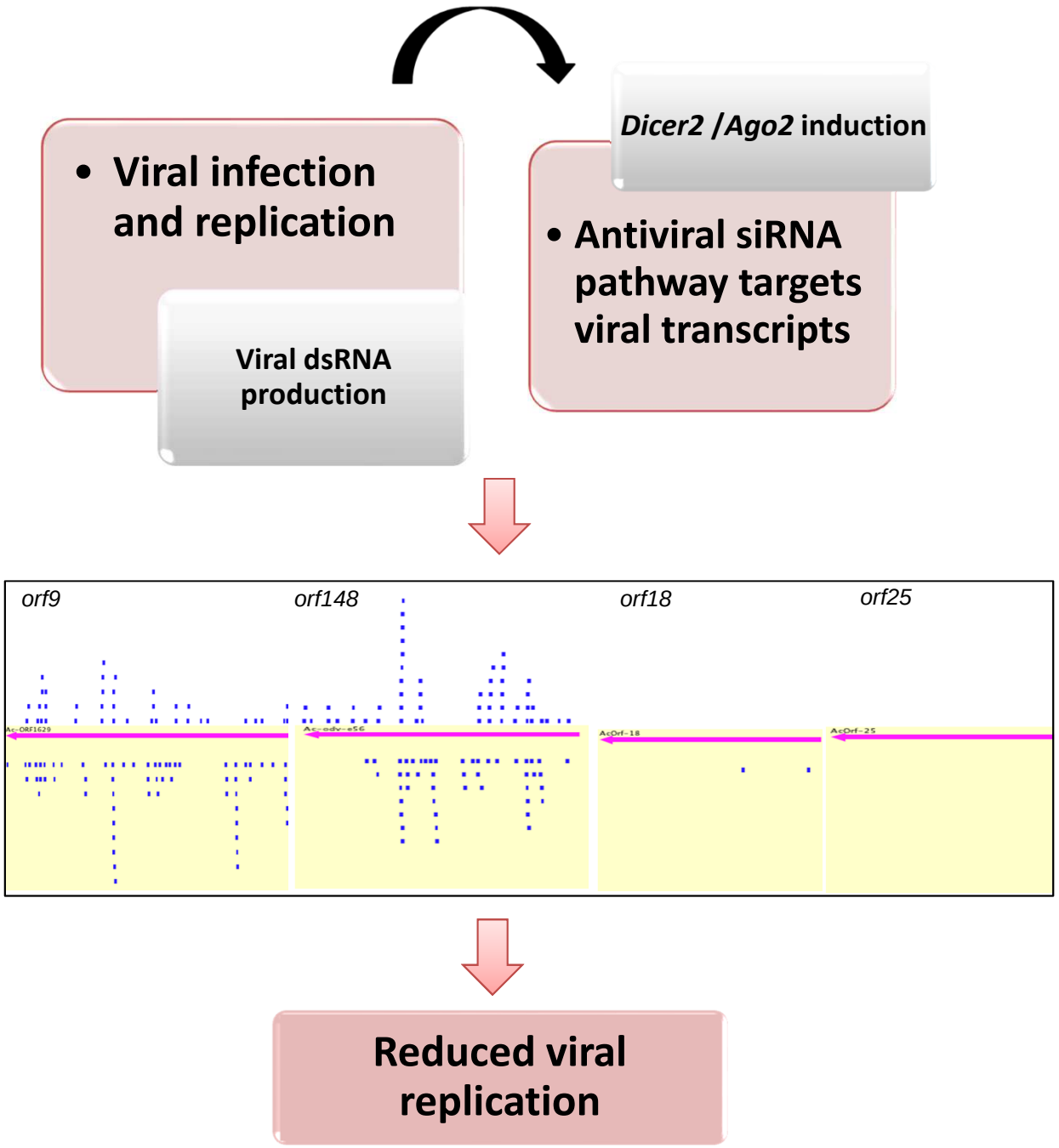
• Viral infection and replication
Viral dsRNA production
Dicer2 /Ago2 induction
• Antiviral siRNA pathway targets viral transcripts
orf9
orf148
orf18
orf25
Reduced viral replication

Small interfering RNA pathway contributes to antiviral immunity in *Spodoptera frugiperda* (Sf9) cells following Autographa californica multiple nucleopolyhedrovirus infection

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Abstract

Autographa californica multiple nucleopolyhedrovirus (AcMNPV) is a well-known virus in the *Baculoviridae* family. Presence of the *p35* gene in the AcMNPV genome as a suppressor of the short interfering RNA (siRNA) pathway is a strong reason for the importance of the siRNA pathway in the host cellular defense. Given that, here we explored the roles of *Dicer-2* (*Dcr2*) and *Argonaute 2* (*Ago2*) genes, key factors in the siRNA pathway in response to AcMNPV infection in *Spodoptera frugiperda* Sf9 cells. The results showed that the transcript levels of *Dcr2* and *Ago2* increased in response to AcMNPV infection particularly over 16 h post infection suggesting induction of the siRNA pathway. Reductions in the expression levels of *Dcr2* and *Ago2* by using specific dsRNAs in Sf9 cells modestly enhanced production of viral genomic DNA which indicated their role in the host antiviral defense. Using deep sequencing, our previous study showed a large number of small reads (siRNAs of ~20 nucleotides) from AcMNPV-infected Sf9 cells that were mapped to some of the viral genes (hot spots). Down-regulation of *Dcr2* in Sf9 cells resulted in enhanced expression levels of the selected virus hotspot genes (i.e. ORF-9 and ORF-148), while the transcript levels of virus cold spots (i.e. ORF-18 and ORF-25) with no or few siRNAs mapped to them did not change. Overexpression of AcMNPV p35 as a suppressor of RNAi and anti-apoptosis gene in Sf9 cells increased virus replication. Also, replication of mutant AcMNPV lacking the *p35* gene was significantly increased in Sf9 cells with reduced transcript levels of *Dcr2* and *Ago2*, highlighting the antiviral role of the siRNA pathway in Sf9 cells. Together, our results demonstrate that *Dcr2* and *Ago2* genes contribute in efficient antiviral response of Sf9 cells towards AcMNPV, and in turn, the AcMNPV p35 suppresses the siRNA pathway, besides being an antiapoptotic protein.

Keywords: Antiviral defense, siRNA, host-virus interactions, small RNAs

1. Introduction

Following virus infection, different cellular immunity pathways such as JAK/STAT, Toll and RNA interference (RNAi) are activated in insects to prevent the spread of viral infection to other cells (Kingsolver et al., 2013). RNAi is well-known as an intracellular conserved mechanism that is critical in gene regulation and cell defense (Ding and Voinnet, 2007). The presence of endogenous or exogenous intracytoplasmic long double strand RNA (dsRNA) can trigger the RNAi machinery including the short interfering RNA (siRNA) or the microRNA pathway in the cell (Ender and Meister, 2010). When a virus infects host cells and starts its transcription and replication, production of intermediate replicative dsRNA and other viral dsRNA structures triggers the siRNA machinery. *Dicer2*, one the most important members of the siRNA pathway, recognizes the viral dsRNA structures and cleaves them to small dsRNAs. *Ago2* then selects one strand of the small dsRNA as a guide virus-derived small interfering RNA (vsiRNA). The guide vsiRNA leads the RNA induced silencing complex (RISC) to target viral RNA molecules that are complementary to the guide vsiRNA (Kingsolver et al., 2013; Lee et al., 2004; Matranga et al., 2005; Mehrabadi et al., 2015; Siomi and Siomi, 2009).

The importance of siRNAs in insect antiviral response was first reported against RNA viruses. It was shown that *Drosophila melanogaster* flies with defects in key component of the siRNA pathway were more sensitive to infection with RNA viruses such as Drosophila C virus (DCV), cricket paralysis virus (CrPV) (van Rij et al., 2006), rhabdovirus vesicular stomatitis virus (Mueller et al., 2010), and Drosophila X virus (Zambon et al., 2006). Also, it was shown that silencing of *Dcr2* and *Ago2* increased the titer of O'nyong'nyong virus in *Anopheles gambiae* (Keene et al., 2004), Sindbis virus (SINV) in *Aedes aegypti* (Campbell et

al., 2008) and Rift Valley fever virus in *Ae. aegypti* and *Culex quinquefasciatus* mosquitoes (Dietrich et al., 2017). The importance of the RNAi pathway in defense against DNA viruses was also investigated. In *D. melanogaster*, mutant flies with defects in the siRNA pathway were more sensitive to infection with Invertebrate iridescent virus 6 (IIV-6) (Bronkhorst et al., 2012). Also, it was shown that silencing of *Dcr2* in *Helicoverpa armigera* facilitated Helicoverpa armigera single nucleopolyhedrovirus (HaSNPV) infection (Jayachandran et al., 2012). As a counter-defense, viruses have evolved different molecular strategies to escape from this potent antiviral defense. Viral suppressers of RNAi (VSR) have been evolved in some viruses to impair the host RNAi machinery. VSRs have been identified in some insect viruses including RNA and DNA viruses, for instance, B2 protein in Flock house virus (Li et al., 2002), CrPV-A1 and DCV-A1 proteins in CrPV and DCV (Nayak et al., 2010), and p35 in Autographa californica multiple nucleopolyhedrovirus (AcMNPV) (Mehrabadi et al., 2015) that are essential for normal infection of the viruses.

Baculoviridae is a family of viruses with a double stranded circular DNA genome ranging from 80 to 200 kbp in length which only replicate in arthropods and mainly have narrow host ranges (Blissard and Rohrmann, 1990; Szewczyk et al., 2009). The most well-known virus in this family is AcMNPV, the type species of the *Alphabaculovirus* genus of the *Baculoviridae* family. Previous studies have suggested the importance of small RNAs in AcMNPV-host interactions. For example, it was shown that microRNA expression is altered in response to AcMNPV infection (Mehrabadi et al., 2013). Moreover, it was also found that the anti-apoptotic *p35* gene of AcMNPV functions as a VSR to attenuate the antiviral RNAi response of the host cells and facilitate virus replication (Mehrabadi et al., 2015). Although these studies highlighted the role of small RNAs, in particular siRNAs, as part of the cellular antiviral mechanism in Sf9 cells, the antiviral role of the siRNA pathway in Sf9 cells still remains to be functionally investigated. In this study, we investigated the response of the

siRNA key components (i.e. *Dcr2* and *Ago2*) following AcMNPV infection. Moreover, their roles in antiviral defense were also investigated. Our results showed that the siRNA pathway was induced following virus infection and played an important role in antiviral defense by attenuating the expression of virus genes and virus replication. Understanding of networks involved in insect-virus interactions would help with the dissection of insect antiviral defense mechanisms and co-evolution of host antiviral defense and virus counter-defense.

2. Methods and materials

2.1 Cells and virus

The *Spodoptera frugiperda* cell line (Sf9) was maintained in SF900-II serum-free medium (Invitrogen) as a monolayer at 27°C. AcMNPV was amplified in Sf9 cells, and budded viruses that accumulated in the medium were used for inoculations. In order to explore the effect of virus infection on expression of *Dcr2* and *Ago2* genes post infection, Sf9 cells were seeded in 12 well plates and infected by 500µl of AcMNPV (MOI of 5) in all experiments.

2.2 Meta-analysis of small RNA deep sequencing and mapping of vsiRNAs

We utilized previously reported small RNA deep sequencing data of mock and AcMNPV-infected Sf9 cells (Mehrabadi et al., 2013; Mehrabadi et al. 2015). Small RNA reads were filtered as previously described, mapped to the AcMNPV genome by using Bowtie mapping software, and visualized by using the Argo genome browser (http://www.broadinstitute.org/annotation/argo/). According to the previously reported small RNA deep sequencing data, there were hot spots and cold spots for vsiRNAs in the AcMNPV genome (Mehrabadi et al., 2015). Among the hot spots of the AcMNPV genome, ORF-9 and ORF-148 were selected, and ORF-18 and ORF-25 were also considered as cold spots for further studies.

2.3 DNA extraction and quantitative PCR (qPCR)

Quantitative PCR was used to determine the levels of viral DNA accumulation in different experiments. To do this, DNA extraction was performed as described before (Glatz et al., 2003) and subjected to qPCR using AcMNPV *ie-1* gene. DNA concentrations were measured by a NanoDrop instrument (BioTek), and 10 ng total genomic DNA was used for each qPCR using SYBR Green Mix without ROX (Ampliqon) with a Mic real-time PCR (BMS) under the following conditions: 95 °C for 15 min, and 40 cycles of 95 °C for 10 s and 60 °C for 45 s, followed by the melting curve (68‑95 °C). Primers used for qPCR are shown in Table 1. *RPL27* was used for normalizing the data. Reactions from three biological replicates were repeated three times.

2.4 RNA extraction and RT-qPCR

Total RNA was extracted from mock and AcMNPV-infected Sf9 cells at 4, 8, 16, 24 and 48 hpi using Tri-Reagent™ according to the manufacturer's instructions (Molecular Research Center Inc.) and subsequently incubated with DNase I at 37 °C for 10 min followed by heat inactivation at 75 °C for 10 min. RNA concentrations were measured using a spectrophotometer and integrity was ensured through analysis of RNA on a 1% (w/v) agarose gel. RT-qPCR was carried out on the RNA extracted from mock and AcMNPV-infected Sf9 cells. Approximately 2 µg of total RNA was used as template in a volume of 20 µl for first-strand cDNA synthesis with oligo (dT) primer. Samples were incubated at 42°C for 60 min followed by heating at 70°C for 5min. The cDNA samples were subjected to qPCR using gene-specific primers (*Dcr2* and *Ago2*) while utilizing *RPL27* as reference (Table 1). QPCR conditions were as follow: 95°C for 15min, followed by 40 cycles of 95°C for 10s, 57°C for 10 s, and 72°C for 20 s, followed by the melting curve (68–95 °C). Transcript levels of four AcMNPV ORFs (ORF-9, -18, -25, and -148) were compared in mock and dsDcr2 transfected Sf9 cells at 24 hpi by RT-PCR utilizing the *RPL27* gene as reference. For each experiment,

three biological replicates with three technical replicates were analyzed.

2.5 RNAi

We used RNAi to silence *Dcr2* and *Ago2* genes in Sf9 cells by specific dsRNA. The specific primers contained a T7 promoter sequences (Table 1) at their 5' end designed for *in vitro* dsRNA synthesis. DsRNAs (~500 bp) were then produced and purified for each fragment using the MEGA Script kit according to the manufacturer's instructions (Ambion). Also, dsRNA of the *green fluorescent protein* gene (dsGFP) was generated as control. Synthesis was confirmed by running dsRNA on an agarose gel and concentrations of dsRNAs were determined using by a nanodrop instrument. To induce RNA silencing *in vitro*, Sf9 cells were resuspended and ~1×10^3 cells added to individual wells of a 12-well plate. Once the monolayers had formed (~1 h) medium was removed and a transfection medium was added. This medium consisted of 0.5 ml SF-900II, 8 µl Cellfectin (Invitrogen), and 2 µg dsRNA either for gene of interest or *GFP*. Twenty four hours after, cells in each well were infected with 200 µl of AcMNPV inoculum as indicated above. The plate was then incubated at 27 °C for 48 h for analyses.

2.6 Assessing the role of AcMNPV *p35* gene as suppressor of RNAi and its effect on virus replication

For expression of the p35 protein of AcMNPV in Sf9 cells, we used a previously constructed vector, pIZ/p35 with OpIE2 promotor (Mehrabadi et al., 2015). The pIZ/p35 vector was transfected into Sf9 cells using Cellfectin reagent (Invitrogen) according to the manufacturer's instructions. pIZ/GFP vector was used as control for transfection of Sf9 cells. Expression of *p35* from the constructs was confirmed by RT-qPCR using a pair of specific primers for *p35* (Table 1). Twenty-four hours after pIZ/p35 transfection, the cells were

infected with AcMNPV. The viral genomic DNA levels were quantified 24 h post infection as described earlier. To question whether the *p35* effect is related to the siRNA suppression, we used a mutant AcMNPV lacking the *p35* gene (Δ^{p35}AcMNPV), which has previously been characterized (Lin et al., 2010), and infected Sf9 cells that were previously transfected with either dsGFP (as control) or specific dsRNA to *Dcr2* and *Ago2* genes. Twenty-four hours after the mutant virus inoculation, virus replication was quantified in the cells using qPCR.

3. Results

3.1 Sf9 cells siRNA pathway was induced upon AcMNPV infection

To assess the effect of AcMNPV infection on induction of the siRNA pathway in Sf9 cells, we analyzed the expression levels of *Dcr2* and *Ago2* genes at different times post infection. Replication of AcMNPV in Sf9 cells at different times post infection was confirmed by qPCR (Fig. 1A). Expression analysis by RT-qPCR showed that the transcript levels of *Dcr2* significantly increased at 8 hpi and reached to the maximum level at 48 hpi (Fig. 1B). *Ago2* expression level was significantly increased at 16 hpi and reached to the highest level at 48 hpi (Fig. 1C). The results suggest that the expression response of *Dcr2* was faster than that of *Ago2* to viral infection (Fig. 1B, C).

3.2 RNAi of *Dcr2* and *Ago2* genes enhanced AcMNPV replication in Sf9 cells

Considering the progressive induction of *Dcr2* and *Ago2* expression levels following AcMNPV infection, we questioned their potential roles in the cell-virus interactions using dsRNA-mediated silencing of these genes. The expression levels of *Dcr2* and *Ago2* were significantly reduced after specific-dsRNA delivery into the cells as shown by RT-qPCR (Fig. 2A). Reduction in the expression of these genes modestly enhanced the replication of

AcMNPV compared to the mock and dsGFP transfected cells (Fig. 2B, C). RNAi of *Ago2* resulted in more than five-fold increase in AcMNPV replication, whereas *Dcr2* RNAi enhanced replication of AcMNPV by two-fold (Fig. 2B, C). There was no difference in virus replication between the control and mock cells.

3.3 RNAi of *Dcr2* gene affects the expression level of some of the AcMNPV ORFs (hot spots)

Virus-derived small interfering RNAs (vsiRNAs) from AcMNPV infected Sf9 cells have been reported previously (Mehrabadi et al. 2015). Uneven distribution of the mapped vsiRNAs on the AcMNPV genome resulted in hot spots (the viral genome regions with large number of mapped vsiRNA highlighting intensive targeting of these regions by siRNA) and cold spots (the viral genome regions with low number of mapped vsiRNA) in the AcMNPV genome. To establish the significance of the siRNA pathway in antiviral response of Sf9 cells, *Dcr2* gene was silenced and its effect on the expression levels of four AcMNPV ORFs was evaluated. AcMNPV ORF-9 and ORF-148 were selected as the hot spot genes, while ORF-18 and ORF-25 were selected from the cold spot genes (Fig. 3A). Our results indicated that RNAi of *Dcr2* significantly increased the transcript levels of the hot spot genes (Fig. 3B, C), while the transcript levels of the cold spot genes were not affected (Fig. 3D, E).

3.4 p35 enhanced replication of AcMNPV through suppression of antiviral RNAi response

We already reported that the AcMNPV *p35* gene acts as a suppressor of RNAi in different animal cells (Mehrabadi et al., 2015). Given that, we used pIZ/p35 to express this viral gene in Sf9 cells and evaluate its effect on AcMNPV replication in the cells. RT-qPCR result showed that *p35* was highly expressed in the Sf9 cells transfected with pIZ/p35 (Fig. 4A).

Expression of *p35* significantly, but moderately, enhanced AcMNPV replication in Sf9 cells as shown by qPCR (Fig. 4B). This modest effect is most likely due to the fact that AcMNPV already expresses p35, but overexpression of the protein prior to viral infection could further enhance viral replication by prompting suppression of the RNAi and apoptotic responses in the cells. Moreover, we used a mutant AcMNPV lacking the *p35* gene (Δ^{p35}AcMNPV) and infected Sf9 cells that were previously transfected with either dsGFP or specific dsRNA to *Dcr2* and *Ago2* genes. Our results showed that specific dsRNA-mediated suppression of *Dcr2* and *Ago2* significantly increased replication of the mutant virus in comparison to the control cells transfected with dsGFP (Fig. 4C, D).

4. Discussion

The small interfering RNA (siRNA) pathway is known as an effective antiviral response in insects against viruses (Blair and Olson, 2015; Bronkhorst et al., 2012; Bronkhorst and van Rij, 2014; Hussain et al., 2016; Jayachandran et al., 2012; Mehrabadi et al., 2015). Antiviral role of the siRNA pathway is evident against different RNA viruses in insects, however, the production of dsRNA, which triggers RNAi, has been demonstrated in both RNA and DNA viruses (Weber et al., 2006). Recent studies have shown the contribution of the siRNA pathway as an antiviral response against DNA viruses as well, although to a lesser extent. RNAi response against a DNA virus was reported in *D. melanogaster* infected with IIV6 where production of siRNA from both strands of the virus genome was evident in *Drosophila* infected cells (Bronkhorst et al., 2012; Kemp et al., 2012). Further, the direct role of *Dcr2* in antiviral response of *Helicoverpa armigera* cells (HzFB) against HaSNPV was also confirmed (Jayachandran et al., 2012). In the present study, we demonstrated the roles of *Dcr2* and *Ago2*, the key components of the siRNA pathway, in antiviral immunity of Sf9 cells.

Our results showed upregulation of *Dcr2* and *Ago2* expression levels following AcMNPV infection. Considering the important roles of these genes in the siRNA pathway, their induction in response to virus infection indicates induction of the cellular siRNA pathway. We found that the expression level of *Dcr2* increased prior to *Ago2* that could be in accordance to their role in the siRNA pathway in which *Dcr2* functions upstream of *Ago2* by cleaving dsRNA into siRNAs. The resultant siRNAs are then utilized by the RISC complex guiding *Ago2* to target specific RNA molecules (Bernstein et al., 2001). Therefore, it seems that *Dcr2* expression is induced upon virus infection to initiate the antiviral siRNA pathway. Consistent to our results, *Dcr2* has been suggested to recognize viral dsRNA generated during replication (Bernstein et al., 2001; Lee et al., 2004; Marques et al., 2013; Soares et al., 2014). Moreover, we showed that RNAi of *Dcr2* and *Ago2* modestly enhanced viral replication suggesting the antiviral role of these genes in Sf9 cells. The effect on virus replication was more pronounced when these genes were silenced in cells infected with p35-null AcMNPV. While these effects may not appear large, they could be sufficient to suppress the RNAi response, which may not be as strong as a response mounted against an RNA virus. Consistently, in other studies, the effect of silencing *Dcr2* and *Ago2* genes on replication of other DNA viruses, IIV6 (Bronkhorst et al., 2012; Kemp et al., 2012) and HaSNPV (Jayachandran et al., 2012), was found to be modest. In AcMNPV, combination of anti-RNAi and anti-apoptotic properties of p35, which are independent of each other, could provide a more potent suppressive effect on various arms of the host cellular antiviral responses. The role of *Ago2* in antiviral immunity has been reported against different RNA viruses such as O'nyong'nyong virus in *An. gambiae* (Keene et al., 2004), dengue virus in *Ae. aegypti* and DCV and CrPV in *D. melanogaster* (van Rij et al., 2006). We also showed that RNAi of *Dcr2* resulted in enhanced expression of viral hot spot genes; however, the expression levels

of cold spot genes were not changed. These results confirmed the antiviral role of the siRNA pathway through targeting transcripts of virus during the infection process.

Given the important role of *Dcr2* and *Ago2* in host antiviral immunity, some viruses encode suppressors of RNAi (VSRs) that interact with these proteins thereby suppressing the host siRNA pathway (Nayak et al., 2010). It was reported that the CrPV lethal and the DCV chronic infections in *D. melanogaster* are related to their VSRs. CrPV-1A protein interacts with *Ago2,* while DCV-1A interfered with *Dcr2* function. Also, infected *D. melanogaster* with a recombinant SINV that encodes CrPV-1A showed more mortality in comparison with flies that were infected with a recombinant SINV encoding the DCV-1A VSR protein (Nayak et al., 2010). It was also demonstrated that the *p35* gene of AcMNPV acts as a suppressor of the host siRNA pathway in different cells (Mehrabadi et al., 2015). To assess the effect of *p35* on Sf9 antiviral response and AcMNPV replication, we overexpressed this gene in Sf9 cells and found that it enhances viral replication, although this could also be partially due to anti-apoptotic function of the p35 protein (Clem, 2007). Using mutant AcMNPV lacking *p35* gene and dsRNA-mediated reduction of *Dcr2* and *Ago2* transcripts, we also showed that the siRNA pathway functions as a potent antiviral mechanism in Sf9 cells.

In the present study, we provide further evidence for the contribution of the siRNA pathway in insect antiviral defense against DNA viruses. We showed that this antiviral pathway is induced in response to AcMNPV, and targets viral transcripts that may form dsRNA due to overlap of negative and positive strand transcripts or secondary structures following transcription. Silencing *Dcr2* and *Ago2* moderately enhanced AcMNPV replication and the transcript levels of hot spot genes. Also, the role of AcMNPV *p35* gene in enhancing viral replication was further confirmed. Considering our results and taking previous reports into account, the involvement of the siRNA pathway in antiviral defense in Sf9 cells is confirmed.

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Figure legends

Figure 1: Induction of the siRNA pathway genes (*Dicer-2* and *Ago2*) of Sf9 cells in response to AcMNPV infection. A) qPCR analysis of DNA from Sf9 cells at various times following AcMNPV infection revealed increases in viral genomic DNA (gDNA) over time. B) Expression of *Dicer-2* increased in response to AcMNPV infection. C) The transcript levels of *Ago2* intensified after AcMNPV infection. In all the experiments, DNA and RNA extractions were performed at 4, 8, 16, 24 and 48 hpi. Asterisks indicate a significant difference between the control and the infected Sf9 cells (Brown-Forsythe test, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

Figure 2: Antiviral role of the siRNA pathway (*Dicer-2* and *Ago2*) in Sf9 cells. A) RT-qPCR analysis confirmed silencing of *Dcr2* and *Ago2* genes using dsDcr2 and dsAgo2 24 and 48 hours post transfection, respectively. B and C) AcMNPV genomic DNA (gDNA) replication increased after reduction in *Dcr2* and *Ago2* transcript levels, respectively, analyzed by qPCR on DNA samples which were extracted from the cells at 24 hpi. *Rpl27* gene was used as reference gene. Asterisks indicate a significant difference between the control and the infected Sf9 cells (Brown-Forsythe test, $^{**}P < 0.01$, $^{***}P < 0.001$).

Figure 3: Effect of *Dcr-2* silencing on selected AcMNPV ORF transcript levels. A) Diagram showing mapping of small RNAs (blue small lines) from deep sequencing of AcMNPV-infected Sf9 cells to hot spot (ORF-9 and ORF-148) and cold spot (ORF-18 and ORF-25) genes in the AcMNPV genome that were selected for analysis (Mehrabadi et al., 2015). White and yellow backgrounds represent the positive and negative viral strands, respectively. B-E) Sf9 cells were transfected with dsDcr2, followed by 5 MOI AcMNPV infection and RT-qPCR analyses, which revealed increases in the transcript levels of the hot spot genes (ORF-9 and ORF-148) but not those of the cold spot genes (ORF-18 and ORF-25)

as compared with mock or dsGFP transfected Sf9 cells. Cell were collected for RNA extraction 24 h post AcMNPV infection. *RPL27* was used as reference gene (Brown-Forsythe test, $^{*}P < 0.05$, $^{**}P < 0.01$).

Figure 4: Baculovirus suppressor of RNAi (p35) enhances AcMNPV replication. A) The expression levels of p35 in Sf9 cells transfected either with pIZ/p35, or infected by AcMNPV, or both. B) The expression of p35 in Sf9 cells slightly increased replication of AcMNPV in Sf9 cells. C) RT-qPCR confirmed decline of *Dicer-2* and *Ago2* genes in Sf9 cells treated with dsDcr2 and dsAgo2 at 24 hours post transfection relative to mock or dsGFP transfected cells before Δ-p35AcMNPV infection. D) qPCR analysis of DNA from Δ-p35AcMNPV-infected Sf9 cells transfected with dsGFP, dsDicer2 or dsAgo2 showed significant increases in the genomic DNA (gDNA) levels of AcMNPV in dsDicer2 and dsAgo2 transfected cells at 24 hpi (Brown-Forsythe test, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{***}P < 0.0001$).

Table 1: Primer sequences used in this study. 414

Primer	Primer sequence (5'− 3')	Fwd./ Rev.	Annealing temp (°C)	reference
Sf_*Ago2*_RNAi-F	TAATACGACTCACTATAGGGGGTTATGAAATGTGGACGGG	Fwd.	60	This study
Sf _*Ago2*_RNAi-R	TAATACGACTCACTATAGGGTTCGATGGGGAAGTAGATGC	Rev.	60	This study
Sf _*Dcr2*_RNAi-F	TAATACGACTCACTATAGGGACAAGGCGACGACAAGACAA	Fwd.	60	This study
Sf _*Dcr2*_RNAi-R	TAATACGACTCACTATAGGGGTACTTAGCAGGAGGCGCAG	Rev.	60	This study
Sf _*Dcr2*_qPCR-F	AAAACTGTACGAGGCCGGAG	Fwd.	60	This study
Sf _*Dcr2*_qPCR-R	TGCCTTGGTGCCTGGATAAG	Rev.	60	This study
Sf _*Ago2*_qPCR-F	CCAAGCGATAAAAGGCACGG	Fwd.	60	This study
Sf _*Ago2*_qPCR-F	AGACACGGATCGCATACACC	Rev.	60	This study
Sf _*RPL27*_qPCR-F	GAAGCCAGGTAAAGTGGTGCT	Fwd.	60	This study
Sf _*RPL27*_qPCR-R	GTGTCCGTAGGGCTTGTCTG	Rev.	60	This study
ORF9-F	ATGACGAATCGTAGATATGAATCTG	Fwd.	60	(Mehrabadi et al., 2015)
ORF9-R	TTAAGCGCTAGATTCTGTGCGTTGT	Rev.	60	(Mehrabadi et al., 2015)
ORF18-F	GGAGGCGCAGCCGTGACATGCCATA	Fwd.	60	(Mehrabadi et al., 2015)
ORF18-R	CGACGACACCAACACATGATATTCG	Rev.	60	(Mehrabadi et al., 2015)
ORF25-F	ATGGCAACTAAACGCAAGATTGGCG	Fwd.	60	(Mehrabadi et al., 2015)
ORF25-R	GTCTTGCCGTTGGCCGGCTCCAAC	Rev.	60	(Mehrabadi et al., 2015)
ORF148-F	ATGAGTTTTTTTTCAAATCTTCGCG	Fwd.	60	(Mehrabadi et al., 2015)
ORF148-R	TTATCGAGGGGCCGTTGTTGGTGTG	Rev.	60	(Mehrabadi et al., 2015)
p35-mid F	CAAAACCCGTTCTCATGATGTT	Fwd.	60	(Mehrabadi et al., 2015)
p35-mid R	GTGAGCAAACGGCACAATAAC	Rev.	60	(Mehrabadi et al., 2015)

Fig.1

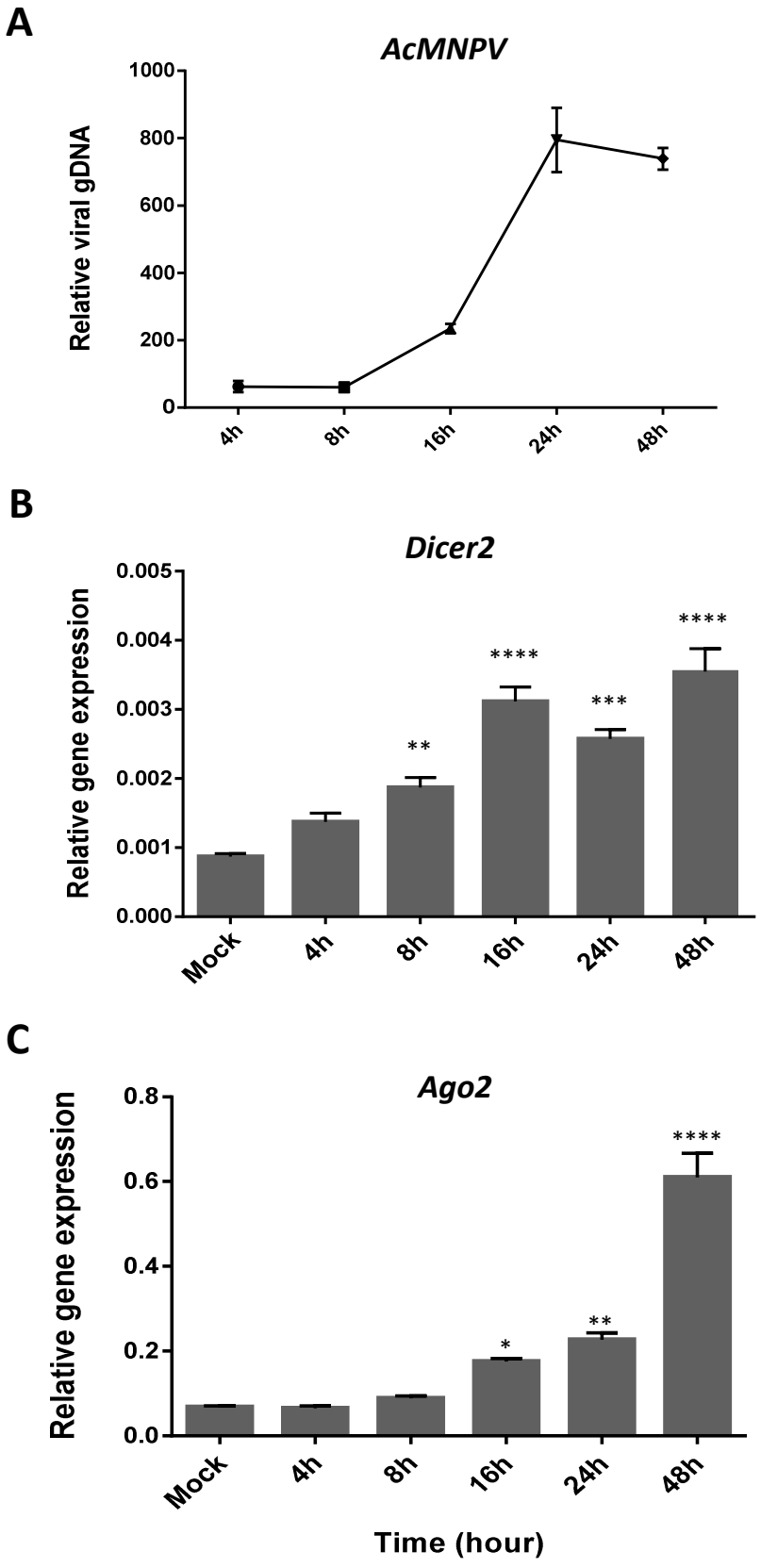

A
AcMNPV
Relative viral gDNA
1000
800
600
400
200
0
4h
8h
16h
24h
48h
B
Dicer2
Relative gene expression
0.005
0.004
0.003
0.002
0.001
0.000
**
****
***
****
Mock
4h
8h
16h
24h
48h
C
Ago2
Relative gene expression
0.8
0.6
0.4
0.2
0.0
*
**
****
Mock
4h
8h
16h
24h
48h
Time (hour)

Fig. 2

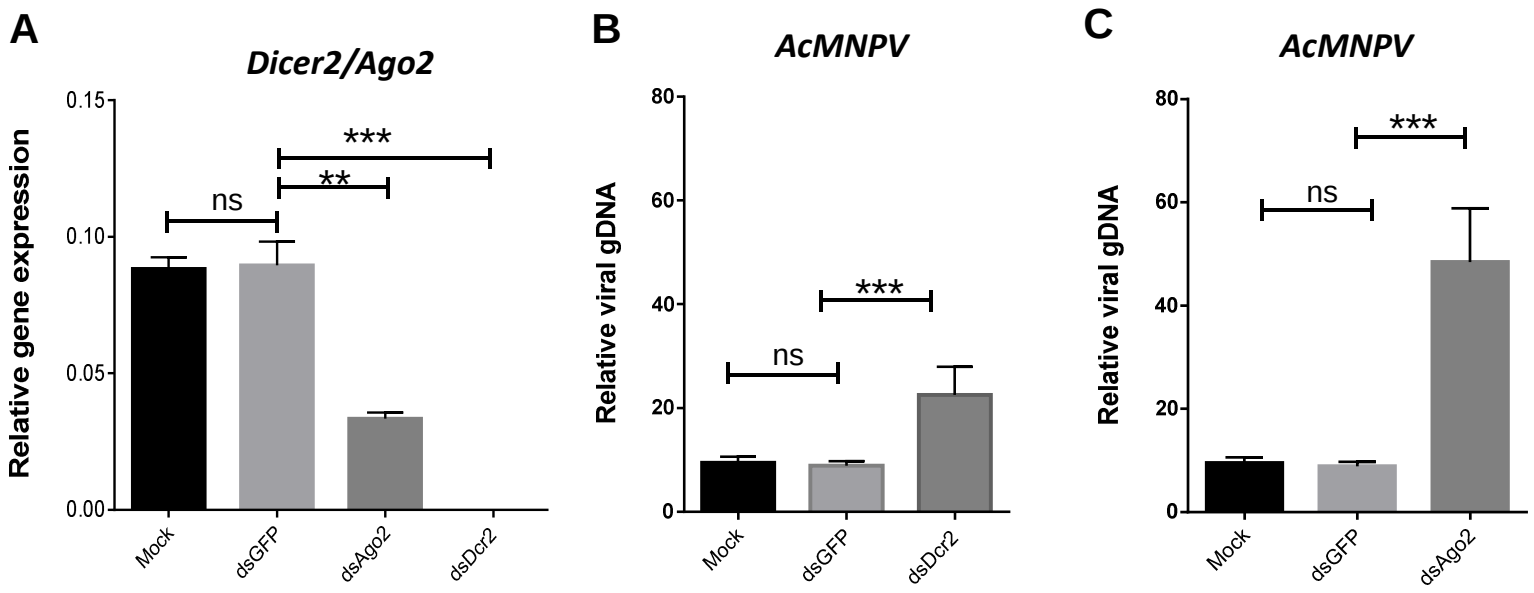

A
Dicer2/Ago2
Relative gene expression
0.15
0.10
0.05
0.00
ns
**
***
Mock
dsGFP
dsAgo2
dsDcr2
B
AcMNPV
Relative viral gDNA
80
60
40
20
0
ns
***
Mock
dsGFP
dsDcr2
C
AcMNPV
Relative viral gDNA
80
60
40
20
0
ns
***
Mock
dsGFP
dsAgo2

Fig. 3

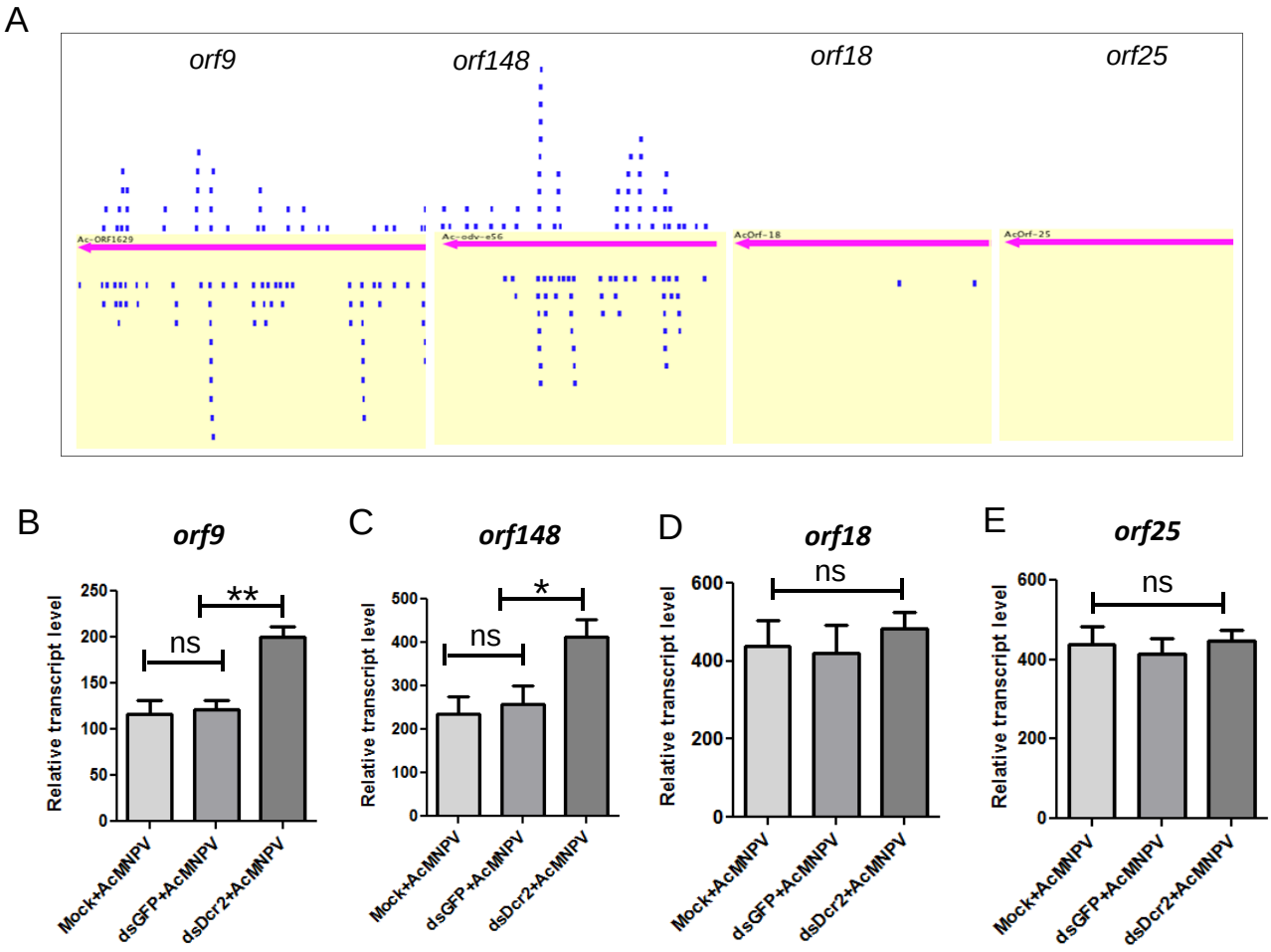
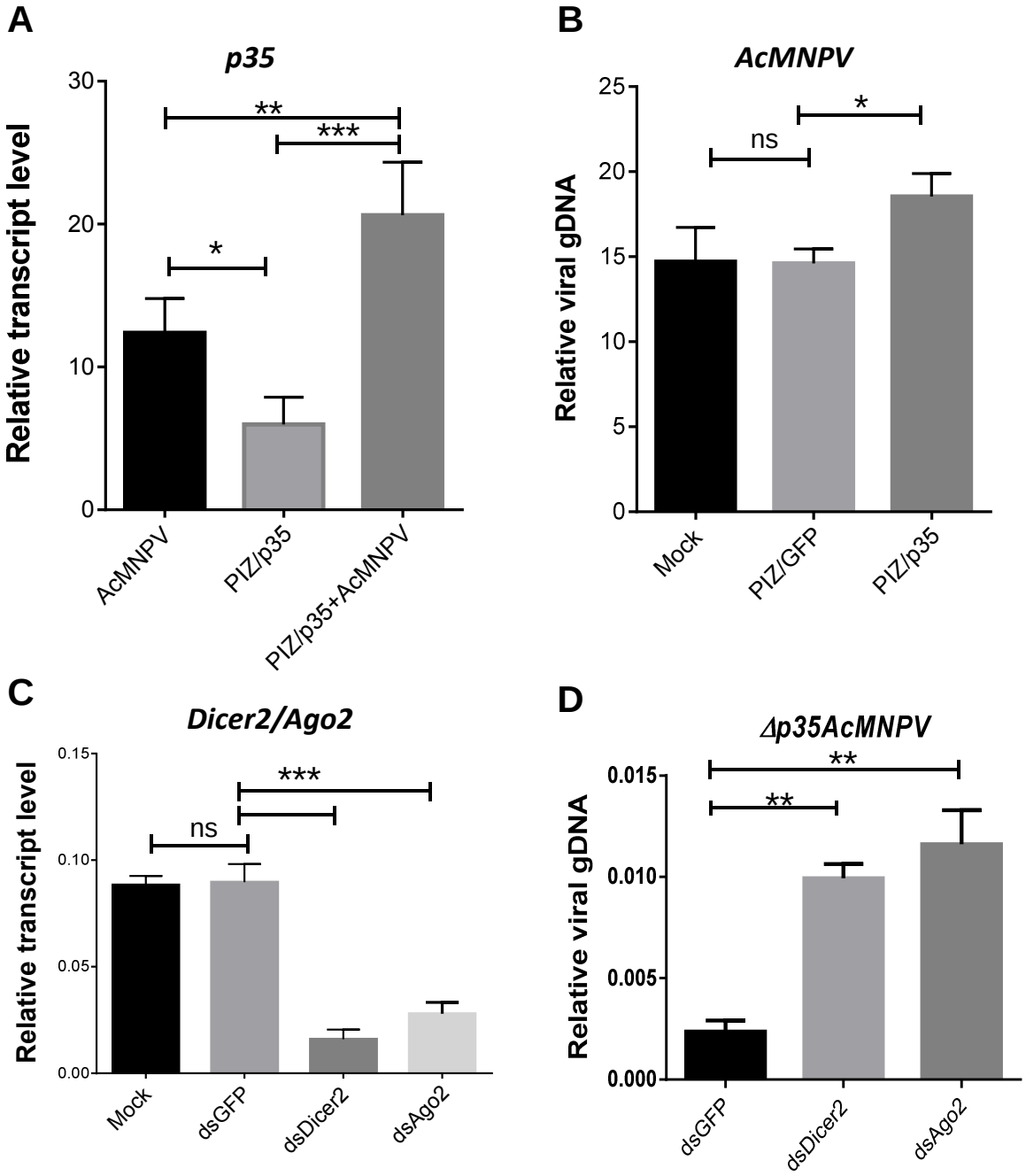

A
orf9
orf148
orf18
orf25
Ac-ORF1629
Ac-odv-e56
AcOrf-18
AcOrf-25
B
orf9
Relative transcript level
250
200
150
100
50
0
**
ns
Mock+AcMNPV
dsGFP+AcMNPV
dsDcr2+AcMNPV
C
orf148
Relative transcript level
500
400
300
200
100
0
*
ns
Mock+AcMNPV
dsGFP+AcMNPV
dsDcr2+AcMNPV
D
orf18
Relative transcript level
600
400
200
0
ns
Mock+AcMNPV
dsGFP+AcMNPV
dsDcr2+AcMNPV
E
orf25
Relative transcript level
600
400
200
0
ns
Mock+AcMNPV
dsGFP+AcMNPV
dsDcr2+AcMNPV

Fig. 4


A
p35
Relative transcript level
30
20
10
0
**
***
*
AcMNPV
PIZ/p35
PIZ/p35+AcMNPV
B
AcMNPV
Relative viral gDNA
25
20
15
10
5
0
*
ns
Mock
PIZ/GFP
PIZ/p35
C
Dicer2/Ago2
Relative transcript level
0.15
0.10
0.05
0.00
***
ns
Mock
dsGFP
dsDicer2
dsAgo2
D
Δp35AcMNPV
Relative viral gDNA
0.015
0.010
0.005
0.000
**
**
dsGFP
dsDicer2
dsAgo2

Highlights

- Small interfering RNA pathway (siRNA) induced in response to AcMNPV infection.
- *Dcr2* and *Ago2* genes have antiviral roles in Sf9 cells.
- The presence of the *p35* gene of AcMNPV enhanced the viral replication through suppression of siRNA pathway.
- SiRNA pathway targets the viral transcript thereby attenuating virus replication.